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IDENTIFICATION OF *ARMILLARIA* SPECIES IN THE ROCKY MOUNTAIN REGION

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ABSTRACT

An investigation was carried out in the USDA Forest Service Rocky Mountain Region to identify the species of *Armillaria* present throughout the Region and to determine their geographic distribution. Vegetative isolates of *Armillaria* spp. were collected from dying and dead ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), pinyon pine (*Pinus edulis*), white spruce (*Picea glauca*), Engelmann spruce (*Picea engelmannii*), Colorado blue spruce (*Picea pungens*), subalpine fir (*Abies lasiocarpa*), white fir (*Abies concolor*), Douglas-fir (*Pseudotsuga menziesii*) and quaking aspen (*Populus tremuloides*) in 11 National Forests located in 21 counties of Colorado, southern Wyoming, and South Dakota during 1991 and 1993. One hundred and four vegetative *Armillaria* spp. isolates were dual-cultured with each other and with known diploid testers representing seven of the nine North American Biological Species (NABS). Only one species, *Armillaria ostoyae* (NABS I), was identified from the field-collected isolates.

INTRODUCTION

It is well known that *Armillaria* spp. cause root diseases on a large number of species of trees, shrubs, vines, and herbs throughout the temperate and tropical regions of the world (Kile *et al.* 1991; Williams *et al.* 1989), living either as parasites or saprophytes (Wargo and Shaw 1985). In the drier, interior region of western North America, *Armillaria* spp. show more aggressive behavior in coniferous forests by attacking, colonizing, and killing healthy trees of all ages (Wargo and Shaw 1985). *Armillaria* spp. cause localized heavy mortality, especially in recently-cut or thinned stands and plantations. It is the most common root disease in the Rocky Mountain Region (Johnson 1985).

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Historically, isolates of *Armillaria* spp. have been identified as belonging to a single species, *Armillaria mellea* (Vahl:Fr) Kummer (Singer 1956). As a result of widely varied ecological associations (Ullrich and Anderson 1978) and the remarkable variation in cultural and fruiting body characteristics (Gibson 1961; Raabe 1966; Singer 1975), *Armillaria mellea sensu lato* has subsequently been studied as a species complex. Several biological species have been identified from *Armillaria mellea sensu lato* in North America (NABS I - *Armillaria ostoyae* (Romagnesi) Herink, NABS II - *A. gemina* Berube & Dessureault, NABS III - *A. calvescens* Berube & Dessureault, NABS V - *A. sinapina* Berube & Dessureault, NABS VI - *A. mellea* (Vahl:Fr.) Kummer, NABS VII - *A. gallica* Marxmuller & Romagnesi, NABS IX and NABS X - unnamed, NABS XI - *A. cepistipes* Velenovsky), Europe, and Australia (Anderson and Ullrich 1979; Anderson 1986; Guillaumin *et al.* 1991).

A variety of methods has been used to identify biological species of *Armillaria*. Techniques used include diploid-diploid mycelial interaction or somatic incompatibility (Adams 1974; Shaw and Roth 1976; Kile 1983; Korhonen 1978; Mallett and Hiratsuka 1986; Worrall 1994; and McDonald *et al.*, in prep.); haploid-diploid mycelial interaction (McDonald, in prep.); haploid-diploid interfertility test (Siepmann 1987; Rizzo and Harrington 1992); haploid interfertility test (Guillaumin *et al.* 1991); occurrence of clamp connections (Larsen *et al.* 1992); isozyme analysis (Lin *et al.* 1989); DNA sequencing (Anderson and Smith 1988); culture morphology (Rishbeth 1986; Guillaumin *et al.* 1988), and polymerase chain reaction (PCR) (Harrington, pers. comm.). The purpose of this study was to identify the species of *Armillaria* present in the USDA Forest Service Rocky Mountain Region, which includes the states of Colorado, Kansas, Nebraska, South Dakota and Wyoming, and determine their geographic distribution. The identification of *Armillaria* species was done using the diploid-diploid mycelial interaction pairings with all previously identified *Armillaria* species testers. *Armillaria ostoyae* and *A. gallica* were used in all tests, *A. ostoyae* because of its widespread occurrence in western North America (Anderson and Ullrich 1979; Anon 1989; Blenis *et al.* 1987; Filip 1989; Hadfield *et al.* 1986; Mallett 1989; McDonald *et al.* 1987; McDonald and Martin 1988; Morrison *et al.* 1989; Omdal *et al.* 1995 ; Proffer *et al.* 1987; Rizzo and Harrington 1992; Wargo and Shaw 1985; and Whitney *et al.* 1989) and *A. gallica,* because of reports of its presence in the Great Lakes and the possibility of its presence in the Northern Rocky Mountain Region (Smith *et al.* 1992; McDonald and Martin 1988).

MATERIALS AND METHODS

Field collection of *Armillaria* spp. isolates

One hundred and four vegetative *Armillaria* spp. isolates were collected from infected woody root materials obtained from dead or dying trees or stumps of ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), pinyon pine (*Pinus edulis*), white spruce (*Picea glauca*), Engelmann spruce (*Picea engelmannii*), Colorado blue spruce (*Picea pungens*), subalpine fir (*Abies lasiocarpa*), white fir (*Abies concolor*), Douglas-fir (*Pseudotsuga menziesii*), and quaking aspen (*Populus tremuloides*) in 11 National Forests located in 21 counties of Colorado, southern Wyoming, and South Dakota during 1991 and 1993 (Table 1). A map showing the counties within which collections were made is presented in Figure 1.

Isolation and pure culture of field isolates

A sterilized increment hammer was used to take subsamples from root specimens. Small pieces (approx. 4 mm in diameter and 2 mm in thickness) of wood, with mycelial fan and cambium intact, were taken for the first isolation attempt (Figure 2). Five to six samples were placed on a basidiomycete selective medium (Figure 3). This medium consisted of 3% malt extract, 2% dextrose, 1.9% agar, 0.5% Bacto peptone, 6 ml of 1% ortho-phenylphenol (OPP) stock solution and 100 ml of 1000 ppm streptomycin stock solution in 900 ml distilled water. Alternative sampling of only mycelial fans on this medium took at least one month to grow to a distinct mycelial colony, whereas the combined wood-mycelia samples grew within 10 days. The combined wood-mycelial fan sample was therefore preferred throughout this experiment.

After 10 days incubation in the dark at room temperature (24°C), a small segment of rhizomorph or mycelial colony was transferred to a malt agar medium (the same medium as used for isolation except opp and streptomycin were omitted) for pure culture.

Pairing tests selected

The diploid-diploid mycelial interaction test (D-D test) was used to identify *Armillaria* species since only diploid isolates were available and both vegetative compatibility group (VCG) delineation and species identification were desired. The D-D test is a pairing method for VCG delineation and species identification by dual-culturing two *Armillaria* isolates on an artificial medium to test their compatibility (McDonald *et al.*, in prep.). For delineation of VCG, two

unidentified isolates are paired against each other; for species identification, an unidentified isolate is paired with a previously identified isolate, or tester. In our experiments, we used NABS diploid testers. Pairing reactions indicate whether the two isolates are of the same VCG, different VCGs within the same species (intraspecific) or of different species (interspecific). Observed reactions include fusion (colonies anastomose with no interaction lines), clear zone (1 - 4 mm clear zone forms between the isolates), or black line (black line of melanized hyphae forms between the isolates). For self-pairings, confluent hyphal growth occurs between the isolates. This delineates isolates that may be of the same VCG. For intraspecific pairs, the colonies grow together to a lesser degree with a clear zone forming between the isolates. For interspecific pairs, a black line of melanized hyphae will form between isolates (Mallett and Hiratsuka 1986; Mallett *et al.* 1989; McDonald *et al.*, in prep.).

Pairings

One hundred and two *Armillaria* isolates were divided into six experimental groups for pairing and identification: E1, E2, E3, E4 E5, and E6 (Table 2). First, field isolates were paired with each other in all possible combinations within each experimental group to delineate VCGs. A representative isolate from each VCG was subsequently paired with the *Armillaria* testers for species identification. Nineteen diploid *Armillaria* testers were used, two or three of these testers were from each of seven *Armillaria* spp. (Table 2). A total of 5,150 pairings were performed to delineate clones and species.

Pairing for VCG

For VCG delineation, the following procedure was used: for each *Armillaria* isolate, two 1-1.5 mm flat squares of subcultures were cut from the pure culture and placed on one side of 3% malt agar medium in a 60x15 mm petri plate (Figure 4). Two subcultures of another isolate were placed on the opposite side of the medium; the two different isolates were placed along the two sides of a trapezoid (McDonald *et al*, in prep.). Each plate was labeled with a randomly generated number and placed in an incubator to grow for 3-4 weeks at room temperature (24°C) in the dark. Two replicates for each isolate-isolate pair were made. The pairings were read independently three times by the first author between 21 and 28 days of growth.

For E1, 17 isolates were paired with each other (Table 2 and Table 3); 15 isolates were used for E2 (Table 2 and Table 4); 16 isolates for E3 (Table 2 and Table 5); 13 isolates for E4

(Table 2 and Table 6); 19 isolates for E5 (Table 2 and Table 7); and 22 isolates were paired with each other for E6 (Table 2 and Table 8). Anastomosing hyphae were the criterion for delineation of VCGs. A representative isolate of each VCG was subsequently used in the D-D test for species identification.

Pairing for species identification

The representative isolates were each paired with a few of the 19 known diploid testers representing seven NABS provided by Dr. Geral McDonald (Table 2).

A dual-culture pairing procedure, similar to that used above, was used for the pairings for species identification. Instead of placing field isolates on both sides of the medium in a petri dish, two subcultures of a field isolate were placed on one side and an *Armillaria* tester on the opposite side.

The number and species of *Armillaria* testers used in the experimental groups were varied based on their importance in the geographical area, results from the previous D-D tests, and personal communication with Dr. McDonald.

For E1 and E2, diploid testers of *A. ostoyae*, *A. gallica*, and NABS X were used. Two replicates for each isolate-tester pair were made and pairing interpretations were performed by three different persons. Only the isolates identified by all three persons were recorded. The unidentified isolates from E1 and E2, after pairing with *A. ostoyae, A. gallica* and NABS X, were paired with *A. gemina*, *A. calvescens*, *A. sinapina* and *A. mellea* diploid testers. Six replicates were made for each isolate-tester pair, and 18 replicates for species identification. *A. ostoyae* was included again in the second run of E1 and E2 because of its importance in this area.

For E3 and E4, only *A. ostoyae*, *A. gemina*, *A. gallica*, and NABS X diploid testers were used since the isolates were unlikely to be *A. calvescens*, *A. sinapina,* or *A. mellea* (Table 7) as determined by the tests E1 and E2.

For E5 and E6, *A. ostoyae*, *A. gemina*, and *A. gallica* were used since the evidence from previous tests indicated that the isolates collected from this area belonged to *A. ostoyae*, with a few ambiguous results from the pairings of isolates with *Armillaria gemina* testers. *A. gallica* was included since it has been reported in surrounding states.

For all the D-D test pairings, except E1 and E2, four replicates were made for each isolate-tester pair and 12 replicates were used for species determination.

Pairing reaction interpretation

To avoid subjective errors, all pairings conducted throughout this study were performed "blind," in which the only information provided to the reader was a randomly assigned number (McDonald *et al.*, in prep.).

Each pairing reaction was read three times and recorded as 0 -- fusion (same VCG), 1 -- black line (different species), 2 -- clear zone or non-colored line (same species, different VCGs), or 5 --(contaminated or poor growth) (Mallett *et al.* 1989; McDonald *et al.*, in prep.).

The average of these three readings was calculated and used for interpreting pairing reactions and identification of *Armillaria* species according to the following rules: "clonal association rules (rule 1): if frequency of fusion is at least 2 out of 3 replications of a known isolate paired with another unknown isolate, then one can conclude the unknown probably belongs to the same clone...; intra NABS rule (rule 2): if frequency of non-colored lines are 45% or more or black lines are 55% or less in a population, at least 12 pairs of a single combination of unknown clones or unknown clone x known NABS, then one can conclude the unknown probably belongs to the same biological species or to the known biological species as the case maybe; inter NABS rule (rule 3): if the frequency of black lines is more than 90% in a population of at least 12 pairs for an unknown clone x known NABS, then one can conclude the unknown probably belongs to different NABS or is not a member of the known biological species..."(McDonald *et al.*, in prep.).

RESULTS

VCG identification

The pairing results from E1 (Table 3) indicated that the 17 isolates represented 15 VCGs; isolates J4 and J16 were from the same VCG (VCG 1-4), and J10 and J17 were from the same VCG (VCG 1-10).

The pairing results from E2 (Table 4) indicated that the 15 isolates represented 11 VCGs; P9 and P10, P12 and P13, P14 and P15, P3 and P16 were from the same VCGs (VCG 2-7, 2-9, 2-10. and 2-11), respectively.

The pairing results from E3 (Table 5) indicated that the 16 isolates represented 14 VCGs; isolates E310 and E312, E315 and E316 were from the same VCGs (VCG 3-10 and VCG 3-14), respectively.

The pairing results from E4 (Table 6) indicated that the 13 isolates represented six VCGs: E42, E43, E44, E45, E46, E48 and E49 were from the same VCG (VCG 4-2), E412 and E413 were from the same VCG (VCG 4-5).

The pairing results from E5 (Table 7) indicated that the 18 isolates (VCG information for E518 was not available; E518 was done in a D-D test as a single VCG 5-9) represented nine VCGs; E52 and E53, E57, E510 and E515, E511, E513 and E514, E58, E59 and E512, E516 and E517, and E519 and E520 were from the same VCGs (VCG 5-2, 5-5, 5-6, 5-7, 5-8, and 5-10), respectively.

The pairing results from E6 (Table 8) indicated that the 22 isolates represented sixteen VCGs; E63 and E65, E66 and E68, E610 and E611, E613 and E614, E616 and E617, and E618 and E619 were from the same VCGs (VCG 6-3, 6-5, 6-8, 6-10, 6-12, and 6-13), respectively.

A total of 72 VCGs were delineated from the 104 field-collected isolates of *Armillaria* spp.

Species identification

From each VCG, a representative isolate was used for the D-D test to identify species. Thus J4 and J10 were randomly chosen from the clonal groups from E1; P9, P12, P14 and P16 were chosen from the clonal groups from E2; and single clone isolates from E1 and E2 for pairing tests with the known diploid testers of *A. ostoyae*, *A. gallica* and NABS X. The pairing results were first read by two individuals, then sent to Dr. McDonald for confirmation. J4, J5, J6, J7, J8, J9, J12, J18 and J19 of E1 and P1, P4, P7, P11, P12 and P16 of E2 were all identified as *A. ostoyae* .

The unidentified isolates from the first run of E1 and E2 were paired with the diploid testers of A. *ostoyae*, *A. gemina*, *A. calvescens*, *A. sinapina* and *A. mellea*. Eighteen replicates were included in each species identification. The pairing results showed strong evidence that all the isolates were *A. ostoyae* except J2 and J10, which had less than 55% black line formation with both *A. ostoyae* and *A. gemina*. Independent molecular tests using PCR showed that J2, J10 and P6 belonged to *A. ostoyae* (Harrington, pers. comm.). P6 was included for confirmation since its percentage (58%) of black line formation was only slightly higher than 55%, the threshold value, when it was paired with NABS X (Table 9).

Isolates E310, E315, E42, and E412, which had been chosen from the VCGs with other single VCG isolates from E3 and E4, were then paired with the diploid testers of *A. ostoyae*, *A.*

gemina, *A. gallica*, and NABS X. Pairing results from E3 and E4 showed very strong evidence that all the isolates belonged to *A. ostoyae* (Table 10). PCR identification was done for E412 and found to be *A. ostoyae*, although the percentage of black line for E412-NABS X pairs showed 59% which was slightly higher than the 55% threshold value.

Isolates E52, E58, E510, E511, E516, and E519, representing their VCGs together with other single VCG isolates from E5 and the isolates E63, E66, E610, E613, E616, and E618 representing their VCGs with other single VCG isolates from E6, were then paired with the diploid testers of *A. ostoyae*, *A. gemina*, and *A. gallica* (Table 11, and Table 12). There was not enough data to interprete the results for E58, E62, E63, and E64 since contamination occurred. D-D test results from E5 and E6 indicated that all the isolates were *A. ostoyae*. Isolates E511, E519, E61, E615, and E620 showed very strong evidence that they belonged to *A. ostoyae* since only 0% to 3% formed a black line when paired with *A. ostoyae* testers.

All 72 VCGs from the 104 field-collected isolates were thus identified as a single species, *Armillaria ostoyae*.

DISCUSSION

VCG delineation results showed that isolates from the same VCG were capable of infecting different tree species in a given area. Isolate E48, belonged to the same VCG (VCG 4-2) as isolates E42, E43, E44, E45, E46, and E49, but instead of infecting ponderosa pine, it infected a blue spruce. Isolates E310 and E312 belonged to the same VCG (VCG 3-10) but infected different host species, lodgepole pine and Engelmann spruce, respectively. Isolates P12 and P13 (VCG 2-9) infected subalpine fir and lodgepole pine; isolates J10 and J17 (VCG 1-10) infected ponderosa pine and quaking aspen, respectively, as did J4 and J16 (VCG 1-4) (Table 1). Isolates E52 and E53 (VCG 5-2) infected lodgepole pine and subalpine fir, respectively; and isolate E618 and E619 (VCG 6-13) infected subalpine fir and Engelmann spruce, respectively. Other studies also have indicated vegetative compatible groups of the same *Armillaria* spp. may infect several tree species. Omdal *et al* (1995) found that two different clones of *A. ostoyae* infected both white pine and ponderosa pine; another clone of the same *Armillaria* species infected Douglas-fir and ponderosa pine. Korhonen (1978) expressed " no distinct specialization by different clones of the same *Armillariella* species to different tree species was found."

Four goups of *Armillaria* isolates representing different collections were independently identified using the PCR method as *Armillaria ostoyae* (Dr. Harrington, pers. comm.): **(1)** E42 was the largest VCG, including seven isolates which were identified as *A. ostoyae* by using the D-D test; **(2)** J2 and J10 were also tested using the PCR method since ambiguous results were obtained from D-D pairing; **(3)** J3, P5, P6, P14, E39, and E412 were PCR tested because fewer than 90% black line existed in the D-D test when paired with testers of *Armillaria* species other than *A. ostoyae*, (75%, 70%, 58%, and 78% black line existed when J3, P5, P6, and P14 paired with *A. gemina* respectively; and 69% and 59% black line existed when E39 and E412 paired with NABSX). A low percentage of black line existed when all of these isolates were paired with *A. ostoyae* (2%, 2%, 4%, 30%, 0% and 0%, respectively); and **(4)** E411 and E414 were tested by using PCR only because of culture contamination during the experiment and because there were no D-D test results available for them. All 11 isolates identified by using the PCR test were *A. ostoyae*. The PCR test supported and confirmed the D-D test results. The D-D test (McDonald *et al.* in prep.) , or somatic incompatibility test (Worrall 1994, Smith *et al,* 1994), or intraspecific antagonism (Adams 1974; Hood and Sandberg, 1987; Rishbeth 1978, 1988, 1991; Rizzo and Harrington 1993; Siepmann 1985; and Kile, 1983, 1986) is the most widely used method (Worrall, 1994). Very clear results are obtained when the isolates belong to *A. ostoyae*.

Only one species, *Armillaria ostoyae* has been identified in the Rocky Mountain Region to date. This may be because A. *ostoyae* is the only *Armillaria* species which is pathogenic to conifers in dry interior western forest (Kile *et al*, 1991; Wargo and Shaw 1985). Since most of the *Armillaria* isolates were collected in the Black Hills National Forest and the National Forests in Colorado, additional collections are needed from northwestern Wyoming, Nebraska, and from National Forests near the border of Colorado and Utah to determine if species other than *Armillaria ostoyae* exist in the Rocky Mountain Region.

Shade house studies indicate that most VCGs of *A. ostoyae* are pathogenic on tree species native to the Rocky Mountain Region (Omdal *et al.* 1995). Additional *in situ* studies are needed on the pathogenicity of *Armillaria* spp. (*Armillaria ostoyae*, in particular) to various tree species native to the Rocky Mountain Region and on the relationship of *Armillaria* spp. to habitat types in order to determine where and on what sites *Armillaria* spp. are likely to present management problems.

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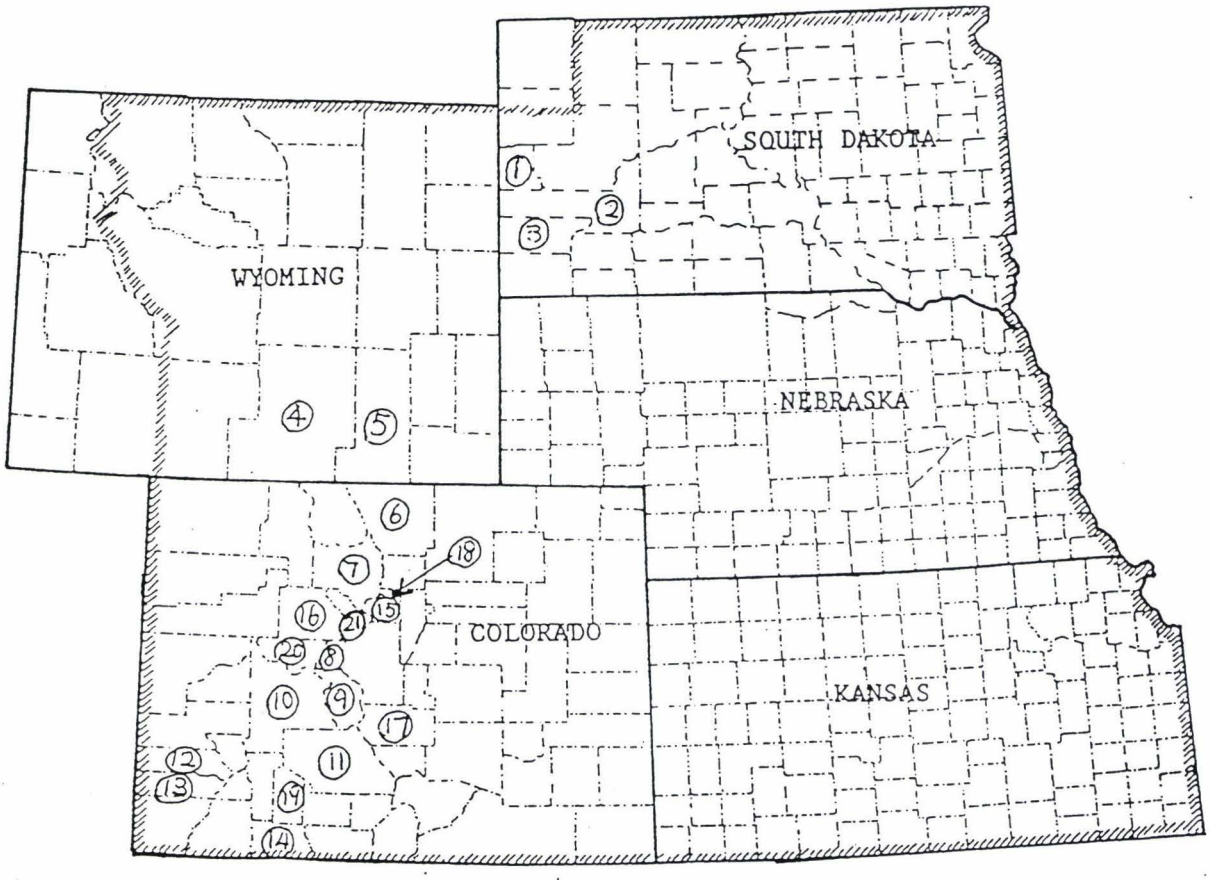

Figure 1. The counties within which *Armillaria* collections were made in the USDA Forest Service Rocky Mountain Region

1) Lawrence County
2) Pennington County
3) Custer County
4) Carbon County
5) Albany County
6) Larimer County
7) Grand County
8) Lake County
9) Chaffee County
10) Gunnison County
11) Saguache County
12) San Miguel County
13) Dolore County
14) Achuleta County
15) Clear Creek County
16) Eagle County
17) Fremont County
18) Gilpin County
19) Mineral County
20) Pitkin County
21) Summit County

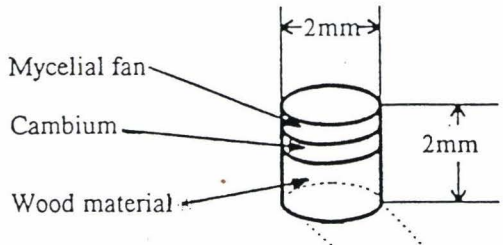

Figure 2. A diagrammatic sketch of Armillaria wood subsample taken by an increment hammer

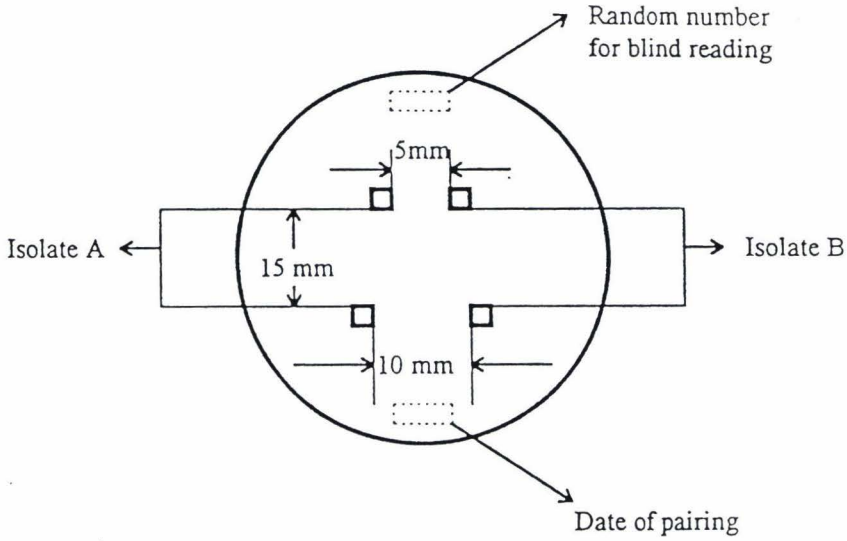

Figure 4. A diagrammatic sketch of D-D mycelial interaction pairing in a 60x15 mm petri dish.

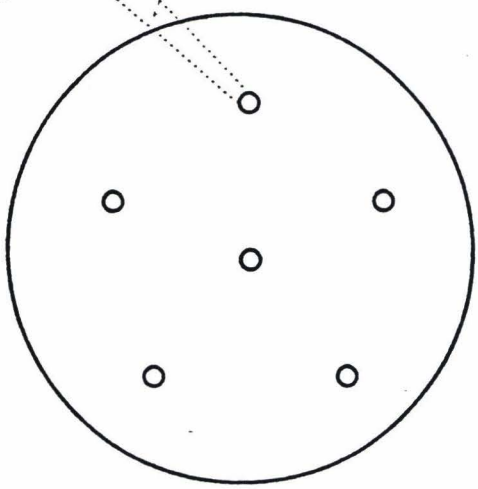

Figure 3. A diagrammatic sketch of 6 combined Armillaria subsamples placed on a selective medium in a 100x15 mm petri dish.

Table 1. *Armillaria* isolates and their identification.

No.	Isolate designation	Location(State/Forest/County)	Host	Species (VCGs)	Methods
1	J1	SD/BH/Lawrence	Ponderosa pine	Armillaria ostoyae (1-1)	D-D
2	J2	SD/BH/Pennington	Ponderosa pine	A. ostoyae (1-2)	D-D/PCR
3	J3	SD/BH/Pennington	Ponderosa pine	A. ostoyae (1-3)	D-D/PCR
4	J4	SD/BH/Lawrence	Ponderosa pine	A. ostoyae (1-4)	D-D
5	J16	SD/BH/Lawrence	Quaking aspen	A. ostoyae (1-4)	D-D
6	J5	SD/BH/Pennington	Ponderosa pine	A. ostoyae (1-5)	D-D
7	J6	SD/BH/Custer	Ponderosa pine	A. ostoyae (1-6)	D-D
8	J7	SD/BH/Lawrence	Ponderosa pine	A. ostoyae (1-7)	D-D
9	J8	SD/BH/Pennington	Ponderosa pine	A. ostoyae (1-8)	D-D
10	J9	SD/BH/Lawrence	Ponderosa pine	A. ostoyae (1-9)	D-D
11	J10	SD/BH/Pennington	Ponderosa pine	A. ostoyae (1-10)	D-D/PCR
12	J17	SD/BH/Pennington	Quaking aspen	A. ostoyae (1-10)	
13	J11	SD/BH/Lawrence	White spruce	A. ostoyae (1-11)	D-D
14	J12	SD/BH/Lawrence	White spruce	A. ostoyae (1-12)	D-D
15	J13	SD/BH/Pennington	White spruce	A. ostoyae (1-13)	D-D
16	J18	SD/BH/Pennington	Ponderosa pine	A. ostoyae (1-14)	D-D
17	J19	SD/BH/Pennington	Ponderosa pine	A. ostoyae (1-15)	D-D
18	P1	WY/MB/Carbon	Lodgepole pine	A. ostoyae (2-1)	D-D
19	P2	WY/MB/Carbon	Subalpine fir	A. ostoyae (2-2)	D-D
20	P4	WY/MB/Albany	Subalpine fir	A. ostoyae (2-3)	D-D
21	P5	CO/BM/Gunnison	Lodgepole pine	A. ostoyae (2-4)	D-D/PCR
22	P6	CO/SI/Chaffee	Pinyon pine	A. ostoyae (2-5)	D-D/PCR
23	P7	CO/UNC/San Miguel	Subalpine fir	A. ostoyae (2-6)	D-D
24	P9	CO/RV/Larimer	Lodgepole pine	A. ostoyae (2-7)	D-D
25	P10	CO/RV/Larimer	Lodgepole pine	A. ostoyae (2-7)	
26	P11	CO/RV/Larimer	Engelmann spruce	A. ostoyae (2-8)	D-D
27	P12	CO/AR/Grand	Subalpine fir	A. ostoyae (2-9)	D-D
28	P13	CO/AR/Grand	Lodgepole pine	A. ostoyae (2-9)	
29	P14	CO/AR/Grand	Lodgepole pine	A. ostoyae (2-10)	D-D/PCR
30	P15	CO/AR/Grand	Lodgepole pine	A. ostoyae (2-10)	
31	P16	CO/AR/Grand	Lodgepole pine	A. ostoyae (2-11)	D-D
32	P3	CO/AR/Grand	Lodgepole pine	A. ostoyae (2-11)	
33	E31	CO/RV/Larimer	Subalpine fir	A. ostoyae (3-1)	D-D
34	E32	CO/RV/Larimer	Lodgepole pine	A. ostoyae (3-2)	D-D
35	E33	CO/SI/Lake	Lodgepole pine	A. ostoyae (3-3)	D-D
36	E34	CO/SJ/Dolores	Spruce	A. ostoyae (3-4)	D-D
37	E35	CO/RG/Saguache	Douglas fir	A. ostoyae (3-5)	D-D
38	E36	CO/SJ/Dolores	Spruce	A. ostoyae (3-6)	D-D
39	E37	CO/SJ/Dolores	Spruce	A. ostoyae (3-7)	D-D
40	E38	CO/SJ/Dolores	Subalpine fir	A. ostoyae (3-8)	D-D
41	E39	CO/AR/Grand	Subalpine fir	A. ostoyae (3-9)	D-D/PCR
42	E310	CO/GU/Gunnison	Lodgepole pine	A. ostoyae (3-10)	D-D
43	E312	CO/GU/Gunnison	Engelmann spruce	A. ostoyae (3-10)	
44	E311	CO/GU/Gunnison	Subalpine fir	A. ostoyae (3-11)	D-D
45	E313	CO/GU/Gunnison	Subalpine fir	A. ostoyae (3-12)	D-D
46	E314	CO/GU/Gunnison	Subalpine fir	A. ostoyae (3-13)	D-D
47	E315	CO/GU/Gunnison	Subalpine fir	A. ostoyae (3-14)	D-D
48	E316	CO/GU/Gunnison	Subalpine fir	A. ostoyae (3-14)	D-D
49	E41	SD/BH/Pennington	Ponderosa pine	A. ostoyae (4-1)	D-D
50	E42	SD/BH/Pennington	Ponderosa pine	A. ostoyae (4-2)	D-D/PCR
51	E43	SD/BH/Pennington	Ponderosa pine	A. ostoyae (4-2)	
52	E44	SD/BH/Pennington	Ponderosa pine	A. ostoyae (4-2)	
53	E45	SD/BH/Pennington	Ponderosa pine	A. ostoyae (4-2)	
54	E46	SD/BH/Pennington	Ponderosa pine	A. ostoyae (4-2)	
55	E48	SD/BH/Pennington	Blue spruce	A. ostoyae (4-2)	
56	E49	SD/BH/Lawrence	Ponderosa pine	A. ostoyae (4-2)	
57	E47	SD/BH/Pennington	Quaking aspen	A. ostoyae (4-3)	D-D
58	E410	SD/BH/Lawrence	Ponderosa pine	A. ostoyae (4-4)	D-D
59	E412	CO/SI/Chaffee	Pinyon pine	A. ostoyae (4-5)	D-D/PCR
60	E413	CO/SI/Chaffee	Pinyon pine	A. ostoyae (4-5)	D-D
61	E415	CO/SI/Chaffee	Pinyon pine	A. ostoyae (4-6)	D-D
62	E411	CO/SI/Chaffee	Lodgepole pine	A. ostoyae	PCR
63	E414	CO/SI/Chaffee	Pinyon pine	A. ostoyae	PCR

Table 1. *Armillaria* isolates and their identification (continued).

No.	Isolate designation	Location(State/Forest/County)	Host	Species (VCGs)	Methods
64	E51	CO/WR/Eagle	Subalpine fir	A. ostoyae (5-1)	D-D
65	E52	CO/RV/Larimer	Lodgepole pine	A. ostoyae (5-2)	D-D
66	E53	CO/RV/Larimer	Subalpine fir	A. ostoyae (5-2)	
67	E54	CO/RV/Larimer	Lodgepole pine	A. ostoyae (5-3)	D-D
68	E55	CO/RV/Larimer	Lodgepole pine	A. ostoyae (5-4)	D-D
69	E57	CO/AR/Clear Creek	Lodgepole pine	A. ostoyae (5-5)	
70	E510	CO/AR/Clear Creek	Lodgepole pine	A. ostoyae (5-5)	D-D
71	E515	CO/AR/Clear Creek	Lodgepole pine	A. ostoyae (5-5)	
72	E58	CO/AR/Clear Creek	Lodgepole pine	(5-7)	
73	E59	CO/AR/Clear Creek	Lodgepole pine	(5-7)	
74	E512	CO/AR/Clear Creek	Lodgepole pine	(5-7)	
75	E511	CO/AR/Clear Creek	Lodgepole pine	(5-6)	
76	E513	CO/AR/Clear Creek	Lodgepole pine	(5-6)	
77	E514	CO/AR/Clear Creek	Lodgepole pine	(5-6)	
78	E516	CO/AR/Gilpin	Lodgepole pine	A. ostoyae (5-8)	D-D
79	E517	CO/AR/Gilpin	Lodgepole pine	A. ostoyae (5-8)	
80	E518	CO/AR/Gilpin	Lodgepole pine	A. ostoyae (5-9)	D-D
81	E519	CO/AR/Gilpin	Lodgepole pine	(5-10)	
82	E520	CO/AR/Gilpin	Lodgepole pine	(5-10)	
83	E61	CO/AR/Clear Creek	Lodgepole pine	(6-1)	
84	E62	CO/AR/Clear Creek	Lodgepole pine	(6-2)	
85	E63	CO/WR/Eagle	Lodgepole pine	(6-3)	
86	E65	CO/WR/Eagle	Lodgepole pine	(6-3)	
87	E64	CO/WR/Eagle	Lodgepole pine	(6-4)	
88	E66	CO/WR/Summit	Lodgepole pine	A. ostoyae (6-5)	D-D
89	E68	CO/WR/Summit	Lodgepole pine	A. ostoyae (6-5)	
90	E67	CO/WR/Summit	Lodgepole pine	A. ostoyae (6-6)	D-D
91	E69	CO/ SI /Fremont	Lodgepole pine	A. ostoyae (6-7)	D-D
92	E610	CO/SI /Fremont	Lodgepole pine	A. ostoyae (6-8)	D-D
93	E611	CO/SI /Fremont	Lodgepole pine	A. ostoyae (6-8)	
94	E612	CO/WR/Pitkin	Spruce	A. ostoyae (6-9)	D-D
95	E613	CO/WR/Pitkin	Subalpine fir	A. ostoyae (6-10)	D-D
96	E614	CO/WR/Pitkin	Subalpine fir	A. ostoyae (6-10)	
97	E615	CO/WR/Pitkin	Subalpine fir	A. ostoyae (6-11)	D-D
98	E616	WY/MB/Carbon	Lodgepole pine	A. ostoyae (6-12)	D-D
99	E617	WY/MB/Carbon	Lodgepole pine	A. ostoyae (6-12)	
100	E618	CO/RG/Mineral	Subalpine fir	A. ostoyae (6-13)	D-D
101	E619	CO/RG/Mineral	Engelmann spruce	A. ostoyae (6-13)	
102	E620	WY/MB/Carbon	Lodgepole pine	(6-14)	
103	E621	CO/SJ /Achuleta	White fir	A. ostoyae (6-15)	D-D
104	E622	CO/GU/Gunnison	Subalpine fir	A. ostoyae (6-16)	D-D

SD - South Dakota, WY - Wyoming, CO - Colorado, BH - Black Hills, MB - Medicine Bow, BM - Blue Mesa, SI - San Isabel, UNC - Uncompahgre, RV - Roosevelt, AR - Arapaho, SJ - San Juan, RG - Rio Grande, GU - Gunnison, WR - White River, D-D - Diploid-diploid mycelial interaction, H-D - Haploid-diploid interfertility test.
PCR - Polymerase chain reaction.

Table 2. Design for *Armillaria* species identification tests of Rocky Mountain Vegetative Compatibility Groups (VGCs). Representative isolates from each VCG were paired with diploid North American Biological Species (NABS) testers in 6 sets of experiments.

Experiment	Numbers of isolates used	Numbers of VCGs used for D-D test	Diploid NABS testers used for D-D test	*Armillaria* spp.
E1	17	15	p1404, st-1, st-2 st-8, st-9, st-11 st-3, st-17, st-18 st-12, M50 st-5, st-20 st-22, st-23, M70 por100, 837, D80	I (*A. ostoyae*) II (*A. gemina*) III (*A. calvescens*) V (*A. sinapina*) VI (*A. mellea*) VII (*A. gallica*) X (unnamed)
E2	15	11	p1404, st-1, st-2 st-8, st-9, st-11 st-3, st-17, st-18 st-12, M50 st-5, st-20 st-22, st-23, M70 por100, 837, D80	I (*A. ostoyae*) II (*A. gemina*) III (*A. calvescens*) V (*A. sinapina*) VI (*A. mellea*) VII (*A. gallica*) X (unnamed)
E3	16	14	p1404, st-1, st-2 st-8, st-9, st-11 st-22, st-23, M70 por100, 837, D80	I (*A. ostoyae*) II (*A. gemina*) VII (*A. gallica*) X (unnamed)
E4	13	6	p1404, st-1, st-2 st-8, st-9, st-11 st-22, st-23, M70 por100, 837, D80	I (*A. ostoyae*) II (*A. gemina*) VII (*A. gallica*) X (unnamed)
E5	19	10	p1404, st-1, st-2 st-8, st-9, st-11 st-22, st-23, M70	I (*A. ostoyae*) II (*A. gemina*) VII (*A. gallica*
E6	22	16	p1404, st-1, st-2 st-8, st-9, st-11 st-22, st-23, M70	I (*A. ostoyae*) II (*A. gemina*) VII (*A. gallica*
Total	102	72		

Table 3. Results of pairings from Experiment 1 (E1) expressed as percent of matings that were vegetatively compatible.

Isolates	J4	J16	J10	J17	J1	J2	J3	J5	J6	J7	J8	J9	J11	J12	J13	J18	J19	VCGs
	% of fusion																	
J4			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1-4
J16			0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	1-4
J10					0	0	0	0	0	0	0	0	0	0	0	0	0	1-10
J17					0	0	0	0	0	0	0	0	0	0	0	0	0	1-10
J1						0	0	0	0	0	0	0	0	0	0	50	0	1-1
J2							0	0	0	0	0	0	0	0	0	0	0	1-2
J3								0	0	0	0	0	0	0	0	0	0	1-3
J5									50	0	0	0	0	0	0	50	0	1-5
J6										0	0	0	0	0	0	0	0	1-6
J7											0	0	0	0	0	0	0	1-7
J8												0	0	0	0	0	0	1-8
J9													0	0	0	0	0	1-9
J11														0	0	0	0	1-11
J12														50	0	0	0	1-12
J13																0	0	1-13
J18																	50	1-14
J19																		1-15

Table 4. Results of pairings from Experiment 2 (E2) expressed as percent of matings that were vegetatively compatible.

Isolate	P3	P16	P9	P10	P12	P13	P14	P15	P1	P2	P4	P5	P6	P7	P11	VCGs
	% of fusion															
P3			0	0	0	0	0	50	0	0	0	0	0	0	0	2-11
P16			0	0	0	0	0	0	0	0	0	0	0	0	0	2-11
P9					0	0	0	0	0	0	0	0	0	0	0	2-7
P10					0	0	0	0	0	0	0	0	0	0	0	2-7
P12							0	0	0	0	0	0	0	0	0	2-9
P13							0	0	0	0	0	0	0	0	0	2-9
P14									0	0	0	0	0	0	0	2-10
P15									0	0	0	0	0	0	0	2-10
P1										0	0	0	0	0	0	2-1
P2											0	0	0	0	0	2-2
P4												0	0	0	0	2-3
P5													0	0	0	2-4
P6													50	0	0	2-5
P7															0	2-6
P11																2-8

Table 5. Results of pairings from Experiment 3 (E3) expressed as percent of matings that were vegetatively compatible.

Isolate	E310	E312	E315	E316	E31	E32	E33	E34	E35	E36	E37	E38	E39	E311	E313	E314	VCGs
	% of fusion																
E310			0	0	0	0	0	0	0	0	0	0	0	0	0	0	3-10
E312			0	0	0	0	0	0	0	0	0	0	0	0	0	0	3-10
E315					0	0	0	0	0	0	0	0	0	0	0	0	3-14
E316					0	0	0	0	0	0	0	0	0	0	0	0	3-14
E31						0	0	0	0	0	0	0	0	0	0	0	3-1
E32							0	0	0	0	0	0	0	0	0	0	3-2
E33								0	0	0	0	0	0	0	0	0	3-3
E34									0	0	0	0	0	0	0	0	3-4
E35										0	0	0	0	0	0	0	3-5
E36											0	0	0	0	0	0	3-6
E37												0	0	0	0	0	3-7
E38													0	0	0	0	3-8
E39														0	0	0	3-9
E311															0	0	3-11
E313																0	3-12
E314																	3-13

Table 6. Results of pairings from Experiment 4 (E4) expressed as percent of matings that were vegetatively compatible.

Isolate	E42	E43	E44	E45	E46	E48	E49	E412	E413	E41	E47	E410	E415	VCGs
	% of fusion													
E42								0	0	0	0	0	0	4-2
E43								0	0	0	0	0	0	4-2
E44								0	0	0	0	0	0	4-2
E45								0	0	0	0	0	0	4-2
E46								0	0	0	0	0	0	4-2
E48								0	0	0	0	0	0	4-2
E49								0	0	0	0	0	0	4-2
E412										0	0	0	0	4-5
E413										0	0	0	0	4-5
E41											0	0	0	4-1
E47												0	0	4-3
E410													0	4-4
E415														4-6

Table 7. Results of pairings from Experiment 5 (E5) expressed as percent of matings that were vegetatively compatible.

Isolate	E51	E52	E53	E54	E55	E57	E510	E515	E511	E513	E514	E58	E59	E512	E516	E517	E519	E520	VCGs
	% of fusion																		
E51	na	na	0	na	0	na	0	0	0	0	0	0	0	0	0	0	na	0	5-1
E52			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5-2
E53				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5-2
E54					0	0	0	0	0	0	0	0	0	0	0	0	0	0	5-3
E55						0	0	0	0	0	0	0	0	0	0	0	0	0	5-4
E57									0	0	na	0	0	0	0	0	0	0	5-5
E510									0	0	0	0	0	0	0	0	na	0	5-5
E515									0	0	0	0	0	0	0	0	0	0	5-5
E511												0	0	0	0	0	na	na	5-6
E513												0	0	0	0	0	0	0	5-6
E514												0	0	0	0	0	0	0	5-6
E58															0	na	0	0	5-7
E59															0	0	0	0	5-7
E512															0	0	0	0	5-7
E516																	0	0	5-8
E517																	0	0	5-8
E519																			5-10
E520																			5-10

Table 8. Results of pairings from Experiment 6 (E6) expressed as percent of matings that were vegetatively compatible.

Isolate	E61	E62	E63	E65	E64	E66	E68	E67	E69	E610	E611	E612	E613	E614	E615	E616	E617	E618	E619	E620	E621	E622	VCGs
	% of fusion																						
E61	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6-1
E62		100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6-2
E63			100	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6-3
E65				100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6-3
E64					100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6-4
E66						100	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6-5
E68							100	0	na	0	0	0	0	0	0	0	na	0	0	0	na	0	6-5
E67								100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6-6
E69									100	0	0	0	0	0	0	0	0	0	0	0	0	0	6-7
E610										100	100	0	0	0	0	0	0	0	0	0	0	0	6-8
E611											100	0	0	0	0	0	0	0	0	0	0	0	6-8
E612												100	0	0	0	0	0	0	0	na	0	0	6-9
E613													100	100	0	0	0	0	0	0	0	0	6-10
E614														100	0	0	0	0	0	0	0	0	6-10
E615															100	0	0	0	0	0	0	0	6-11
E616																100	100	0	0	0	0	0	6-12
E617																	100	0	0	0	0	0	6-12
E618																		100	100	0	0	0	6-13
E619																			100	0	0	0	6-13
E620																				100	0	0	6-14
E621																					100	0	6-15
E622																						100	6-16

Table 9. Results expressed as percent of pairings showing adversion to known *Armillaria* species from diploid-diploid mycelial interaction (D-D) for E1 and E2 isolates.

Isolates	J1	J2	J3	J10	J11	J13	P2	P5	P6	P9	P14
	% of black line										
A. ostoyae	29	19	2	0	6	2	2	2	4	8	30
A. gemina	80	39	75	7	91	89	90	70	58	92	78
A. calvescens	69	78	100	94	84	96	96	92	100	98	91
A. sinapina	92	92	100	100	100	100	100	100	100	92	100
A. mellea	83	89	100	100	100	100	100	100	100	100	100

Table 10. Results expressed as percent of pairings showing adversion to known *Armillaria* species from diploid-diploid mycelial interaction (D-D) for E3 and E4 isolates.

Isolates	E31	E32	E33	E34	E35	E36	E37	E38	E39	E310	E311	E313	E314	E315	E41	E42	E47	E410	E412	E415
	% of black line																			
A. ostoyae	0	5	5	0	0	3	0	8	0	3	5	0	0	0	3	0	0	3	0	3
A. gemina	92	94	97	97	89	100	97	94	100	100	92	100	100	100	100	100	100	100	100	95
A.gallica	100	100	100	100	100	100	88	100	91	100	100	100	100	100	100	100	100	100	97	100
NABS X	100	97	100	100	78	94	97	100	69	100	100	100	100	100	100	100	100	100	59	100

Table 11. Results expressed as percent of pairings showing adversion to known *Armillaria* species from diploid-diploid mycelial interaction (D-D) for E5 isolates.

Isolates	E51	E52	E54	E55	E510	E511	E516	E518	E519
	% of black line								
A. ostoyae	33	0	0	0	3	3	0	0	0
A. gemina	57	97	96	97	79	*	87	70	*
A. gallica	88	94	77	97	82	*	94	100	86

* Results inconclusive due to contamination of cultures.

Table 12. Results expressed as percent of pairings showing adversion to known *Armillaria* species from diploid-diploid mycelial interaction (D-D) for E6 isolates.

Isolates	E61	E66	E67	E69	E610	E612	E613	E615	E616	E618	E620	E621	E622
	% of black line												
A. ostoyae	4	3	0	33	3	0	0	3	3	3	0	0	0
A. gemina	*	71	100	100	85	63	89	75	97	82	*	97	100
A. gallica	87	67	96	77	97	100	100	*	92	82	78	100	100

* Results inconclusive due to contamination of cultures.

Appendix

PCR identification of Armillaria isolates received from Dr. Thomas C. Harrington (Department of Plant Pathology, Iowa State University)

Number	Isolate	IGS Product	AluI fragments	NdeI fragments	HindII fragments	Matching Pattern	Identification
25	J2	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae
26	J3	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae
27	J10	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae
28	P5	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae
29	P6	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae
30	P14	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae
31	E39	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae
32	E42	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae
33	E411	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae
34	E412	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae
35	E414	920	310, 200, 135	550, 370	NT	A. ostoyae	A. ostoyae